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Neurobiology: Sixth Meeting of the European Society  
for Neurochemistry

Claire E. Zomzely-Neurath

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## NEUROBIOLOGY: SIXTH MEETING OF THE EUROPEAN SOCIETY FOR NEUROCHEMISTRY

### 1 INTRODUCTION

The Sixth meeting of the European Society for Neurochemistry (ESN) was held in Prague, Czechoslovakia from 1 through 6 September. There were 850 participants from 32 countries with the largest representation from Western and Eastern European countries. The distribution was unusual in that scientists from Eastern European countries constituted 43 percent of the total number of attendees with the greatest number from the USSR and Czechoslovakia. This was due to the fact that the conference was held in an Eastern European country. In general, conferences held in Western European countries are attended by only a few participants from Eastern European countries.

The format of the ESN meetings consists of a plenary lecture dedicated to the presentation of the state of the art in one area of neurobiology, and symposia of which at least one is focused on a subject of major interest to clinicians. In addition, there are workshops and roundtables on specialized topics as well as poster presentations. At each meeting, two or three young scientists chosen before the meeting present their research as the ESN honorary lectures.

The publishing houses of John Wiley and Sons (Chichester, UK) and Academia (Prague) will jointly publish two volumes with articles based on the lectures given in the Symposia, Workshops, and Roundtables. The titles of these volumes will be *Metabolism and Development of the Nervous System* and *Synaptic Transmitters and Receptors*, reflecting the themes of the ESN meeting in Prague. It is anticipated that these publications will be available in 6 months to 1 year.

A wide range of topics was covered in the symposia, workshops, round tables, and poster sessions (see Appendix I) with English as the obligatory language for all scientific presentations. The topics included molecular biological approaches to studies of the nervous system, neuropeptides and neurotransmitters

and their receptors, membrane lipids and proteins, neurotoxins, immunological approaches, neurogenesis, and neuropathology as well as other research areas.

Because of the large numbers of scientific presentations as well as concurrent sessions, this report encompasses selected topics which deal with research areas of fundamental importance for neurobiologists and are being actively pursued by European neuroscientists. In contrast to the ESN meeting in 1984 (ONRL report C-7-84), there was a great deal of emphasis on the use of molecular biological and immunological techniques.

### 2 PLENARY AND HONORARY LECTURES

The main plenary lecture was presented by E.A. Barnard (MRC Molecular Neurobiology Unit, MRC Center, Cambridge, UK). He dealt with a relatively new and exciting area of research in neurobiology, namely the cloning of genes for receptors for neurotransmitters and neurohormones utilizing the immensely powerful methods of modern molecular genetics. Cloning of the DNA's which encode the receptor subunits can provide the full amino acid sequences, an attainment generally far beyond the range of conventional approaches to protein sequencing. These clones can also yield radiolabeled hybridization probes for analyzing the development and regulation of receptors at the messenger RNA (mRNA) levels, as well as for tracing the chromosomal and cellular locations of their gene expression.

In his excellent lecture, Barnard reviewed the various stages required for the application of the recombinant DNA (rDNA) approach to a receptor protein. The first stage is generally at the level of protein biochemistry, involving purification, subunit analysis and separation, and acquisition of the other fundamental information on the protein. The second stage, for the most usual case of extremely rare mRNA's encoding these proteins is the detection and concentration of those messages. The *Xenopus* oocyte system for cellular translation of receptor mRNA's offers great advantages for

that study and can provide new information in its own right on the receptor. Barnard presented data from his own research and collaborating laboratories on the muscle and brain nicotinic acetylcholine receptor, the brain  $\gamma$ -aminobutyric acid (GABA) receptor and a brain opiate receptor.

The final stages involve the interpretation of the primary structure in terms of the organization of the receptor in the neuronal membrane. Also important at this stage of interpretation is the experimental manipulation of the cloned DNA, for example, to delete or replace individual subunits in the structure or to mutate sites implicated in the receptor function as well as to vary transduction elements. The *Xenopus* oocyte system is again an important tool here in analyzing the requirements for channel function. Thus, the combined results of gene cloning, hybridization tracing, and experimental gene modification are providing a whole new dimension of insights into the nature and function of neuronal receptor systems.

In another plenary lecture, M.J. Berridge (Unit of Insect Neurophysiology and Pharmacology, Department of Zoology, University of Cambridge) discussed another relatively new and important research area, namely receptor-stimulated inositol phospholipid hydrolysis and neural function. Many transmitters in the brain act by stimulating the hydrolysis of an inositol lipid ( $\text{PIP}_2$ ) to give diacylglycerol (DG) and inositol 1,4,5-trisphosphate ( $\text{Ins } 1,4,5\text{P}_3$ ). These two second messengers mark the beginning of a highly versatile signalling system which, according to Berridge, may have a unique role to play in modulating neural activity. By mobilizing calcium from intracellular stores,  $\text{Ins } 1,4,5\text{P}_3$  may regulate the intracellular level of calcium by adjusting the endoplasmic reticular calcium set-point, thereby affecting both excitability and facilitation. The DG/C-kinase pathway, through its ability to modulate a variety of physiological processes, may regulate both transmitter release and excitability. Some of the changes in excitability seem to depend

upon changes in potassium permeability. According to Berridge, receptor-stimulated inositol lipid hydrolysis may thus play a central role in neural function by modulating transmitter release through subtle alterations in excitability.

In an ESN honorary lecture, A.H. Futerman (Department of Neurobiology, Weizmann Institute of Science, Rehovot, Israel) presented data suggesting that acetylcholinesterase (AChE) may be bound to the plasma membrane via a direct interaction with one or more molecules of phosphatidylinositol (PI) and that the hydrophobic anchor of the enzyme is the 1, 2-diacylglycerol moiety of the PI. AChE--the enzyme catalyzing the breakdown of the important neurotransmitter, acetylcholine--exists in a number of molecular forms which can be distinguished by their solubility characteristics and sedimentation coefficients. In the electric organ of *Torpedo marmorata* used by Futerman for his studies, much of the AChE exists as a catalytic subunit characterized by solubilization by detergents (DSACH). This form of the enzyme in *Torpedo* can also be selectively and specifically solubilized by the use of a phosphatidylinositol-specific phospholipase C (PIPLC). Futerman found that at least three other proteins of widely differing origin and function--the Thy-1 antigen of neurons and thymocytes, the variable surface glycoprotein of the parasite *Trypanosoma brucei*, and mammalian alkaline phosphatase--are also attached to the plasma membrane via covalently bound PI as was found for *Torpedo* AChE. The hydrophobic membrane anchoring domain of all these proteins seems to share a number of common structural features. Thus, Futerman thinks that covalent modification by PI may be an ubiquitous mechanism for the anchoring of membrane proteins.

### 3 SYMPOSIA

#### The Impact of Molecular Genetics on Neurochemistry

This session was attended by a very large number of participants, indicating the increased interest of neuroscientists

in the application of molecular biological techniques to neurobiological research.

#### Nicotinic acetylcholine receptor.

Some of their work on the analysis of cloned DNA encoding nicotinic acetylcholine receptor subunits from the chicken central nervous system (CNS) was presented by M.G. Darlison, V.B. Cockcroft, A.A. Hicks, M.D. Squire, S.J. Moss, and E.A. Barnard (MRC Molecular Neurobiology Unit, MRC Center, University of Cambridge Medical School). These investigators are examining the existence and structure of nicotinic acetylcholine receptors in the brain by the use of rDNA technology. DNA fragments encoding subunits of the chick peripheral nicotinic acetylcholine receptor (AChR) were used to probe chick optic lobe complementary DNA (cDNA) libraries in a search for homologous brain sequences. Recombinants identified in this manner were characterized by nucleotide sequencing of cloned inserts. As expected, the deduced neural sequences were found to exhibit strong homology with published peripheral acetylcholine receptor subunit sequences. However, significant differences were observed, particularly in the region between the transmembrane segments M3 and M4. In addition, a chick genomic clone was isolated by cross-hybridization at high stringency, using an optic lobe cDNA clone. An analysis of the nucleotide and primary sequences of these clones was presented.

Regulation of the Tubulin Multigene Family. Some of the continuing excellent research at the Department of Neurobiology, The Weizmann Institute of Science, Rehovot, Israel was presented by U.Z. Littauer on the regulation of the tubulin multigene family. Microtubules are particularly important in the brain where they are involved in cell differentiation, migration, and synaptic transmission. In mammalian DNA, hybridization experiments with labeled tubulin cDNA probes have revealed the presence of  $\alpha$ - and  $\beta$ -tubulin multigene families. However, the number of functional genes is unknown and, indeed, several rat  $\alpha$ -tubulin pseudogenes (nonfunctional) were

identified and sequenced. Specific cDNA probes were constructed to study the expression of an individual member of the tubulin gene family. Two  $\alpha$ -cDNA clones were isolated and their nucleotide sequence was determined. These two clones were found to share high homology within the coding region. However, the 3'-untranslated coding regions are highly divergent. A strong interspecies homology exists in the 3'-untranslated region when compared with specific  $\alpha$ -tubulin isotype sequences from other mammals. The nucleotide sequence of a rat brain  $\beta$ -tubulin shows a high homology when compared to chicken and human  $\beta$ -tubulin sequences. However, the 3'-terminal coding end shows a high degree of divergence and no homology is observed at the 3'-untranslated region. Comparison of the derived amino acid sequences from different species demonstrates that the amino acid changes are not randomly distributed, but rather that there are several conserved and two highly variable regions common to  $\beta$ -tubulin polypeptides from various sources. Three  $\beta$ -tubulin mRNA species are present in rat brain; a dominant neuronal 1.8 kilobase (kb) species and two minor species of 2.6 and 2.9 kb respectively which are developmentally regulated. Recent *in situ* hybridization studies on brain sections have shown that the level of tubulin expression is different in various cells of the cerebellum. Furthermore, immunohistochemistry studies demonstrate that different microtubules are localized in individual cerebellar cells.

Catecholamines: A Molecular Genetic Study. A molecular genetic approach to the study of catecholamines was presented by J. Mallet (Laboratoire de Neurobiologie Cellulaire et Moléculaire, Centre National de la Recherche Scientifique, Gif-sur Yvette, France). Mallet, who is an extremely productive and first class young scientist emphasizing the use of rDNA techniques in neurobiology, discussed some of his continuing work on catecholamines. These compounds are an important class of neurotransmitters present both in the central and peripheral nervous system. They constitute a useful model for the analysis of the

mechanisms involved in the regulation of gene expression during development. Tyrosine hydroxylase (TH), the rate-limiting enzyme in the synthesis of catecholamine has been intensively investigated because of its key role in the physiology of adrenergic neurons. The regulation of its expression is under developmental control, and its synthesis can be induced *in vivo* by nerve stimulation or by treatment with reserpine or steroids. Also, multiple kinase activities may be involved in the short-term regulation of catecholamine biosynthesis by afferent activity.

The entire amino acid sequence of rat and human TH was deduced from cDNA clones. Sequence comparison with phenylalanine hydroxylase indicates that these enzymes share a high degree of homology. A cDNA containing the complete coding sequence of rat TH mRNA was inserted into the SP6 vector system. Microgram amounts of TH mRNA could then be produced. After microinjection into *Xenopus* oocytes, this mRNA was found to direct the synthesis of a protein that has the ability to direct the synthesis of dopa from tryptophan. Thus, the post-translational modifications which are required for the full activity of the enzyme can now be analyzed.

As a model to study the trans-synaptic induction of the enzyme, rats were injected with a single dose of reserpine and the time course changes of both TH mRNA and enzyme activity were analyzed in rat adrenals, locus coeruleus, and substantia nigra. In both locus coeruleus and adrenals, reserpine caused an increase of TH mRNA which was maximal 2 days after drug injection. This increase is about twice that of the enzyme activity. No change was observed in substantia nigra. The effect lasted longer in locus coeruleus than in adrenal. According to Mallet, the time course difference between locus coeruleus and adrenals is most likely to result from a difference in the stability of TH mRNA in the two structures.

Because of the great heterogeneity of nervous tissue, it is desirable that specific mRNA hybridization be detected at the cellular level. Thus, Mallet car-

ried out *in situ* hybridization experiments on adrenal and rat brain.

Mallet has also isolated the TH nuclear gene from a rat cosmid library, and analyzed and introduced this gene by the calcium phosphate precipitate method into mouse neuroblastoma and hamster glial cells that do not produce detectable levels of TH mRNA. He found that transcription and translation of the TH gene occurred in the transfected cells. This approach will be very useful in assessing the functional role of sequences preceding the coding portion of the gene.

The human TH gene was assigned to chromosome 11. Digestion of human cellular DNA with restriction endonuclease *ECOR*I revealed a high frequency restriction fragment length polymorphism which constitutes a suitable marker for future linkage studies involving the TH gene.

Cloned genes for molluscan neuropeptides. Studies involving the use of rDNA methods in nonmammalian systems was discussed by E. Vreugdenhil and J. Joose (Department of Molecular Neuroendocrinology, Biological Laboratory, Free University, Amsterdam, The Netherlands). These investigators cloned genes for molluscan neuropeptides which specify behavioral patterns.

The nervous system of several molluscs can be used as a convenient experimental system for the investigation of cellular and molecular levels, the rates of peptides as neurotransmitters, and their role in the regulation of behavior. Other groups have studied these problems in considerable detail in the peptidergic bag cell neurons of the marine mollusk *Aplysia californica* and in the neurosecretory caudo-dorsal cells (CDC) of the fresh water snail *Lymnaea stagnalis* which control and coordinate egg-laying and accompanying egg-laying behavior in these two species. Vreugdenhil and Joose have used *Lymnaea stagnalis* for their studies.

They have found that CDC release at least nine peptides which are derived from a common precursor of 35 kilodaltons (kD). The best characterized of this peptide set is the caudo-dorsal cell hormone (CDCH). This 36-amino-acid peptide

induces ovulation, stimulates synthesis of secretory products in the female accessory sex glands, and affects identified neurons in the neuronal feeding network.

Because neuropeptides, as a rule, are synthesized as parts of larger precursor proteins, their structure and organization on the precursor are directly approachable by the use of molecular genetic techniques. Using such techniques, Vreugdenhil and Joose were able to isolate and characterize several cDNA clones encoding CDCH and CDCH-like peptides. Southern blotting experiments gave 4 to 5 hybridization signals, suggesting, as in *Aplysia*, the existence of a family of genes which control egg-laying and egg-laying behavior.

Comparison of the CDCH precursor protein with the egg-laying hormone (EGL) of *Aplysia* showed that homology could be found in EGL and the  $\alpha$ ,  $\beta$ , and  $\gamma$  bag cell peptide regions. This homology, however, falls off dramatically in the parts outside this region.

*In situ* hybridization experiments in combination with immunocytochemistry and Northern blotting showed that the members of the CHCH gene family are expressed in a variety of neural and nonneural tissues. Vreugdenhil and Joose are studying the role of this diverse expression in egg-laying behavior.

Acetylcholine Receptors in Locusts. Some excellent studies on insect neuronal transmitter receptors and encoding nucleic acids, in another nonmammalian system (locust) were reported by H. Breer, D. Benke, W. Hawke, and L. Wiczorek (Faculty of Biology, University of Osnabrück, West Germany).

Considerable efforts have recently been made towards a molecular identification of neuronal transmitter receptors. The CNS of insects contains a high concentration of nicotinic acetylcholine receptors (AChR's) and studies have been carried out to identify the neuronal AChR from locusts. Breer et al. purified a large complex receptor protein which appeared to be composed of 4 to 5 identical or very similar polypeptides. When reconstituted in planar lipid bilayers, the

native homooligomeric protein gave a functional ion translocating system activated by cholinergic agonist, thus proving that the protein represents a functional AChR. Immunological approaches revealed that there are obviously significant molecular similarities between the constituents of the neuronal insect receptor and the peripheral heterooligomeric vertebrate receptor. This was confirmed when the N-terminal amino acid sequences of an insect receptor polypeptide fragment was determined. As a first step towards an application of rDNA techniques, RNA preparations from locust nervous tissue were probed for receptor-specific mRNA using reticulocyte lysates and *Xenopus* oocytes as expression systems. Oocytes microinjected with insect poly A<sup>+</sup> RNA produced neuronal AChR polypeptides, which were even inserted into the oocyte surface membrane and displayed specific binding of  $\alpha$ -toxins. Ion flux studies have provided evidence that the binding sites induced represent ACh-gated ion channels and functional AChR's.

Breer et al. constructed a cDNA library using the poly<sup>+</sup> RNA isolated from the nervous tissue of young locusts. The cDNA was cloned into the  $\beta$ -galactosidase structural gene *lac z* of the bacteriophages  $\lambda$  gt 11 which promotes synthesis of fusion proteins. The  $\lambda$  gt 11 recombinant DNA library was screened with specific AChR antibodies. Positive clones were plaque purified and lysogens were made in *E. coli*/1089. Subjecting lysate of the lysogens to SDS-PAGE, followed by immunoblotting, demonstrated the production of a fusion protein that interacts with antibodies to both *E. coli*  $\beta$ -galactosidase as well as locust AChR. The phage DNA has been isolated and the DNA insert subcloned into M13 vector for sequence analysis.

#### Molecular Signaling for Growth and Differentiation in the CNS

Growth Factors in the Nervous System. The topic of growth factors localized in neurons regulating proliferation and maturation of rat astroglial cells in culture was presented by B. Pettmann, G.



Labourdette, F. Perraud, and M. Sensenbrenner (Centre de Neurochimie du CNRS and INSERM U 144, Strasbourg, France). The talk was given by Sensenbrenner, who is well-known for her research on nervous system growth factors. For her studies, she used astroglial cells derived from cerebral hemispheres of newborn rats grown first for 5 days in a serum-containing medium and then switched to a chemically defined medium. The addition of a crude rat or bovine brain extract or of a partially purified fraction to the culture medium at day 5, and again at each medium change, induced a morphological change of the astroglial cells, which became fibrous with elongated processes. The proliferative activity of the astroblasts as well as the biochemical maturation of the cells was enhanced. Two active factors, named astroglial growth factors (AGFS: AGF2 and AGF1) were purified from bovine brain after two and three chromatographic steps, respectively. The purified AGF1 is an acidic protein (PI:5.5) with an apparent molecular weight of about 17,500 daltons. The AGF2 is a basic protein (PI:9.5) of 18,500 daltons. The comparison of the physicochemical properties, the amino acid composition, and the amino-terminal sequences of the AGF's with other growth factors isolated from the brain and affecting the proliferation of other cell types has indicated that AGF2 is identical to the basic fibroblast growth factor (FGF) and AGF1 to the acidic FGF.

Both factors stimulate the proliferation of the cells. Morphological changes are induced, but are not identical. In the presence of the acidic FGF, most cells remain flat with several large and few long thin processes. Under the effect of the basic FGF the cell bodies retracted and became smaller, and many long and thin processes developed.

Polyclonal and monoclonal antibodies against FGF were prepared and used for immunocytochemical localization of this molecule in the rat brain. FGF is found exclusively in neuronal cells. Most neuronal cell bodies are stained and in many areas, the processes are also labeled. The intensity of the staining decreases

with age. The observations that FGF is localized *in vivo* in neurons and is able *in vitro* to induce various effects on astroglial cells suggest strongly that this molecule may play an important role in neuronal-glial cell interaction in the CNS.

A. Prochiantz (Collège de France, INSERM U 114, Paris) presented interesting data suggesting that astrocytes synthesize specific local neuronotrophic factors. He has found that the morphological development of mesencephalic dopaminergic (DA) neurons is regulated by the anatomical region of the monolayer of astrocytes on which they are seeded. Immunocytochemical and ultrastructural studies showed that the growth of the dendritic arbor of the neuron is accelerated on local mesencephalic as opposed to striatal astroglia. Conversely, striatal neurons mature faster on striatal than on mesencephalic astrocytes. These findings have led Prochiantz to propose that specific interactions between neurons and astrocytes from the same region (homotopic associations) are important in the full neuronal maturation.

#### GABA and Neuronal Differentiation.

Some aspects of their continuing and interesting studies on the role of the inhibitory neurotransmitter GABA, as a signal for neuronal growth and differentiation were presented by A. Schousboe, C.H. Hansen, B. Bellhage, and E. Meier (Department of Biochemistry A, The Panum Institute, University of Copenhagen, Denmark). During early development, neurons in the CNS undergo an extensive differentiation during which they develop their respective synaptic machinery; i.e., neurotransmitter enzymes, release processes, and receptors. These differentiation processes are known to be subject to regulation by different external factors and one such factor appears to be GABA. Schousboe et al. have shown that *in vivo* in the brain as well as in retina and in cultured neurons exposure of neurons to GABA may lead to an enhancement of neuronal differentiation both at the morphological level and the functional level. They have shown also that the development of synaptic structures and

subcellular structures associated with protein synthesis and axonal transport is enhanced by GABA or GABA-receptor-specific agonists. Likewise, the development of GABA receptors is greatly enhanced by treatment of different neural tissues and cultures with GABA or GABA agonists. The mechanism by which this neurotrophic activity is mediated is largely unknown but Schousboe thinks that it almost certainly involves interaction with GABA-receptors on the neuronal plasma membrane.

#### Gangliosides and Neurotropic Agents.

Studies of gangliosides as neuromodulatory and neurotrophic agents for neurons of the CNS were reported by W. Seifert, A. Wieraszko, H.J. Fink, F. Förster, and M. Hollmann (Department of Neurobiology, Laboratory of Molecular Neurobiology, Max Planck Institute for Biophysical Chemistry, Göttingen, West Germany). These studies are of particular interest since almost all the work on gangliosides as neurotrophic agents has been done on nerve cells of the peripheral nervous system or neuroblastoma cells in culture. For their studies on functional aspects of gangliosides in the CNS, Seifert et al. have used the rat hippocampus as a model system at four different levels:

1. In a dissociated serum-free cell culture system (embryonic rat)
2. In the hippocampal slice preparation (adult rat)
3. During reactive synaptogenesis after lesion of entorhinal cortex
4. In synaptic plasma membranes from rat cortex and from hippocampus.

They used the hippocampal cell culture system to investigate the possible neurotrophic effects of individual gangliosides on pyramidal neurons both in terms of survival and in terms of neurite outgrowth. They also studied--in addition to exogenous ganglioside application--the endogenous pattern of gangliosides during *in vitro* differentiation of these neurons. Both these strategies were also applied in the *in vivo* studies of the regenerating hippocampus following entorhinal lesions.

In addition, Seifert et al. studied the functional role of gangliosides in synaptic transmission and synaptic plasticity with the hippocampal slice preparation. Their results suggested a special role for the monosialo-ganglioside (GM1) in glutaminergic transmission. By using a glutamate binding assay with synaptic plasma membranes from cortex and hippocampus, Seifert et al. were able to demonstrate a stimulation of glutamate binding to a specific receptor type by gangliosides. In both cases, ganglioside-calcium complex formation appeared to be functionally involved in the mechanism of action.

#### 4 WORKSHOPS

##### Biology of Glial Cells

Several different types of studies were presented in this workshop, including the use of rDNA techniques.

Peripheral Nerve Myelin. Studies of the biosynthesis of the major integral protein of peripheral nerve myelin, the Po protein *in vitro* were reported by P.J. Brophy, C.S. Gillespie, and L. Bernier (Department of Biological Sciences, University of Stirling, UK) and D.R. Colman, D.D. Sabatini (Department of Cell Biology, New York University Medical Center, New York). Peripheral nerve myelin is known to be made in Schwann cells, analogous to oligodendrocytes (glial) cells of the CNS. They found that the Po protein is synthesized on membrane-bound polysomes and is cotranslationally inserted into the endoplasmic reticulum. The N-terminal signal sequence is removed and core glycosylation occurs at the rough endoplasmic reticulum. Myelin basic proteins are synthesized on free polysomes in close proximity to the growing myelin process. By contrast, P2 mRNA's are not enriched in this myelin-associated pool of polysomes. The 2, 3-cyclic nucleotide phosphohydrolases (Wolfgram proteins) are also peripheral proteins of the myelin membranes. They are synthesized on free polysomes but like the P2 protein their mRNA's are not enriched in those polysomes associated with myelin.

**Myelin Proteolipid Protein.** The molecular cloning of mRNA coding for rat brain myelin proteolipid protein (PLP) was presented by A. Dautigny, P.M. Alliel, M.G. Mattei, D. Pham-Dinh, and P. Jolles (Protein Laboratory, CNRS, UA 118, University of Paris V, France). A cDNA library was constructed from rat brain mRNA in pBR322 and screened with a 14-mer mixed oligonucleotide probe. A positive clone containing a 1334 base pair (bp) insert was isolated and sequenced. The cDNA encoded information for the 276 amino acids of rat PLP. PLP was found to be highly conserved during evolution: only three differences were found between the deduced rat PLP sequence and the human PLP sequence recently established by conventional protein sequence methods. The initiator methionyl codon immediately precedes the NH<sub>2</sub>-terminal glycine, indicating that PLP does not require a signal peptide sequence for insertion into the myelin membrane.

Using *in situ* hybridization with PLP cDNA probe, the PLP gene was localized on human chromosome X at band q22 and on mouse chromosome X at band F1. This localization may have implications for x-linked human myelin disorders. In mouse, Dautigny et al. provided evidence that the sex-linked recessive mutation, *jimpy*, is located in the structural gene coding for PLP.

**Dopaminergic Neurons.** S. Denis-Donini (Department of Biology, University of Milan, Italy) presented some intriguing studies on neuronal responses to glial signals. The shape of a neuron, which is the basis of the highly specific connectivity characterizing the mature brain, seems to be determined by a combination of extrinsic and intrinsic factors. In order to understand the relative contribution of nature and nurture, Denis-Donini studied *in vitro* the behavior of two sets of neurons from mouse embryos: (1) the afferent dopaminergic (DA) neurons from the substantia nigra and (2) the local circuit DA interneurons from the olfactory bulb (OB). Denis-Donini had previously shown that nigral afferent neurons have the capacity to respond to various environmental signals.

By contrast, interneurons from the OB have a fixed morphology; i.e., short axons and dendrites that never change in all conditions tested. This striking difference between both sets of neurons was best exemplified by comparing them cultured on OB glia. The interneurons remained unmutated, whereas afferent neurons extended gigantic processes. This latter effect was correlated with the exceptional capacity of the OB to sustain the regeneration of primary sensory axons into a CNS environment and can be due to the fact that astrocytes and superficial glia in the bulb synthesize large amounts of laminin that is deposited in the extracellular matrix. Therefore, laminin seems to be a choice substrate for stimulating process outgrowth in CNS afferent neurons but not in interneurons. These results show that two sets of neurons, although sharing the same neurotransmitter, responded differently to environmental signals in a way that reflects their functional phenotype. This suggests, according to Denis-Donini that neuron-glia interactions might occur through an effector-receptor mechanism.

**Molecular Cloning of a Glia-Derived Neurite-Promoting Factor.** Exciting results on the molecular cloning of a glia-derived neurite-promoting factor (GdNPF) were presented by S. Gloor, J. Guenther, H. Nick, R. Meier, J. Sommer, and D. Monard (Friedrich Miescher Institute, Basel, Switzerland). The GdNPF, a 43-kD polypeptide, had been purified to homogeneity from medium conditioned by C6 rat glioma cells. Gloor et al. have found that this factor is a potent serine protease inhibitor which forms a sodium dodecyl sulfate-resistant complex with proteases such as urokinase, tissue plasminogen activator, thrombin, and trypsin. Gloor et al. have constructed a cDNA library from C6 glioma mRNA. A rat cDNA clone with a sequence coding for GdNPF was isolated by hybridization-selected transcription using rabbit anti-GdNPF polyclonal antibodies. The first cDNA clone was used for screening a second rat glioma cDNA library having larger inserts. The amino acid sequence from rat

GdNPF has been deduced from the nucleotide sequence of the isolated cDNA clone. The identity of this sequence has been confirmed by gas-phase sequencing of some tryptic fragments from the purified GdNPF protein. Northern blot analysis indicated a postnatal regulation of the GdNPF gene during rat brain development.

#### Neurotransmitters and Peptides in Stress

The aim of this workshop was to analyze the role of brain and peripheral catecholamines—nonadrenaline, adrenaline, and dopamine; of brain serotonin and histamine; and of enkephalins in neuroendocrine processes under stress in the rat and man. Understanding of the exact mechanisms of the stress response appears to be considerably complicated by the fact that monoaminergic and peptidergic systems interact in the control of neuroendocrine responses under stress.

#### Noradrenergic System and Stress.

Studies of the brain noradrenergic system in stress were discussed by T.I. Belova and P.K. Anokhin (Institute of Normal Physiology, USSR Academy of Sciences, Moscow, USSR). Belova had shown previously that the locus coeruleus (LC) plays an important role in blood pressure regulation under emotional stress, that stress increased the permeability of the blood brain barrier (BBB), and that ruptures of brain parenchymal vessels occurred, especially in the formatio reticularis mesencephali (FR mes). Belova has found that LC also has a regulatory influence on BBB function and the integrity of brain vessels. Since the FR mes is the structure involved in many kinds of adaptive functions (homeostatic and behavioral) it is suggested that emotional stress leads to a deficiency of the organism's adaptive function. Belova proposed the following mechanism of disturbances of adaptive function under stress: emotional stress → LC hyperfunction → changes in brain energy metabolism → ruptures of brain parenchymal vessels, and as the consequence of these events, → damage of nerve and glial cells (in FR mes especially) → disturbance of the organism's adaptive capacity.

#### Serotonergic System and Stress.

Studies of another transmitter system in stress, namely the serotonergic system were reported by J. Culman, F. Zeman. (Institute of Experimental Endocrinology Bratislava, Czechoslovakia) and C.C. Chiueh (National Institute of Mental Health, Bethesda, Maryland). Turnover of brain serotonin (5-HT) reflects the functional state of the 5-HT system. In general, increased activity of 5-HT neurons accelerates the biosynthesis of 5-HT, its release, and its degradation. 5-HT biosynthesis depends on the availability of the precursor, tryptophan (TP), its uptake in 5-HT nerve terminals, and the rate of hydroxylation of TP by tryptophan hydroxylase (TPH). The activation of TPH increases the biosynthesis of 5-HT. The rate of 5-HT degradation depends on the release of 5-HT and its reuptake into the nerve endings and/or glial cells. 5-HT after its release and reuptake is more susceptible to conversion to 5-hydroxyindolacetic acid (5-HIAA) by monoamine oxidase.

All these factors are involved in the regulation of the 5-HT system activity in stress conditions. Culman et al. found that acute stress increases 5-HIAA levels in the majority of examined brain areas. Increased TP levels and increased accumulation of 5-hydroxytryptophan after decarboxylase inhibition in several brain areas suggest the accelerated synthesis of 5-HT, according to Culman et al. These results demonstrate the increased turnover of 5-HT in the brain, presumably due to an increase in the release of 5-HT from the nerve terminals during acute stress.

#### Corticotropin Releasing Factor and Vasopressin.

In a different approach, the effect of insulin-induced hypoglycemia on the turnover of corticotropin releasing factor (CRF) and vasopressin (VP) in the median eminence (ME) of the rat was reported by F. Berkenbosch and P.J.H. Tilders (Department of Pharmacology, Medical Faculty, Free University, Amsterdam). The study was based on the known effect of insulin-induced hypoglycemia to stimulate pituitary adrenocorticotropin (ACTH) secretion in animals and

man. In the present study, groups of fasted Wistar rats were decapitated at different time-intervals after administration of insulin. CRF and VP were measured in the external of ME by quantitative immunocytochemistry or ME-extracts were made for radioimmunoassay of CRF. Insulin had no marked effects on the VP and CRF concentration in the ME. Administration of colchicine in a dose that blocks axonal transport in VP-producing neurons had no effect on VP and CRF content of the ME in unstressed rats. However, additional administration of insulin caused a time-related decline of both VP and CRF in the ME to 40 to 50 percent after 3 hours without affecting the glucose and ACTH response as seen in noncolchicine treated rats. Pretreatment of colchicine-treated rats with dexamethasone blocked the ACTH responses and prevented the hypoglycemia-induced reduction of VP and CRF in the ME. Thus, it appears that (a) hypoglycemia enhances the secretory activity and thereby the turnover of CRF and VP neurons terminating in the ME and (b) VP and CRF released from their nerve terminals are rapidly replenished by fast axonal transport.

Catecholamines, Cortisone, and Stress. The response of brain catecholamines and plasma corticosterone to peripheral viral injection was reported by A.J. Dunn and M.L. Powell (Department of Neuroscience, University of Florida, Gainesville). In intact mice, they found an increase in plasma corticosterone concentration following the interperitoneal injection (i.p.) of Newcastle Disease Virus (NVD). In contradiction to previous reports from other laboratories, no increase was seen in hypophysectomized animals. However, Dunn and Powell did observe significant increases in catecholamine catabolites in the brains of both intact and hypophysectomized mice after injection of NVD. These results suggest that the brain responds to an antigenic challenge (stress) to the immune system and may involve immune defense mechanisms.

Neural Induction of Enzymes During Stress. Studies of the central regula-

tion of the sympathoadrenal system in animals under stress were presented by T. Sourkes (Department of Psychiatry, McGill University, Montreal, Canada). The phenomena of neural induction of enzymes in rats subjected to stress has been used to identify and locate the site of some CNS neurotransmitters involved in regulation of specific functions of the adrenal gland. Thus, Sourkes has found that induction of TH is favored by activation of a dopaminergic system in the A9 region of the brain and is retarded by serotonergic fibers emanating from the medial raphe nucleus. In contrast, dopamine  $\beta$ -hydroxylase (DBH) induction, which also occurs in the stressed rat, entails reduction of both catecholamines and serotonin, as seen in the reserpinized animal or those given a combination of  $\alpha$ -methyltryptamine and p-chlorophenylalanine. DBH, like TH, is also inducible through a central muscarinic action (oxotremorine); this induction is antagonized under the influence of GABA agonists.

Pituitary Response to Stress. A detailed study on the role of the adrenomedullary-pituitary axis in the control of the pituitary response to stress was presented by F.J.H. Tilders, F. Berkenbosch, and I.D. van Zoest (Department of Pharmacology, Medical Faculty, Free University, Amsterdam). Tilders et al. found that peripheral administration of epinephrine (EP) at doses resulting in plasma EP levels as seen during stress, increases the circulating concentration of various stress-like hormones (prolactin [PRL], ACTH,  $\beta$ -endorphin [END], a melanocyte-stimulating hormone [MSH]) to levels seen during stress. These effects appear to be mediated by  $\beta$ -adrenoreceptors and are of physiological significance since the acute increase of some pituitary hormones (e.g., PRL, MSH, END, but not of ACTH) during exposure of rats to emotional stressors was found to be attenuated by pretreatment of rats with  $\beta$ -adrenergic blockers. Also, pretreatment of rats with chorisondamine, a blocker of stress-induced secretion of EP and norepinephrine, activated the MSH, END, and PRL responses to emotional stressors. In addition, adrenal enucleation, which

results in a disappearance of stress-induced secretion of PI, reduced the PRL, MSH, and END responses to novel environment and footshock. Tilders et al. think that their observations show that catecholamines released from the adrenal medulla are involved in the release of hormones from lactotroph and melanotroph cells during emotional stress.

Opioid Peptides and Stress. C.R. Van Loun, K. Pierzchaen, L. Brown, S. Moua, P. Zeman, and R. Kvetnansky (Department of Medicine, University of Kentucky and V.A. Medical Center, Lexington, Kentucky, and Institute of Experimental Endocrinology, Center for Physiological Sciences, Slovak Academy of Sciences, Bratislava, Czechoslovakia) reported on an *in vivo* study of stress-induced secretion of sympathoadrenal opioid peptides and catecholamines in two animal models (rats and dogs). They found that stress (immobilization) stimulated the secretion of proenkephalin A-derived opioid peptides in both animal models. Repeated stress in the rat appeared to be associated with adaptation of the Met-enkephalin (Met-Enk) response with decreased release but increased synthesis or processing of adrenal Met-Enk.

#### Immunological Approaches to Synapse and Development of the Nervous System

Immunological Detection of Transmitters. A.C. Cuervo (Department of Pharmacology, McGill University, Montreal, Canada), who has been applying hybridoma technology to investigate transmitter-specific neurons in the central and peripheral nervous system, has developed two new methods for application to such studies. Cuervo and others have found that there seems to be an intrinsic cross-reactivity of monoclonal antibodies (Mabs) which results in a discrepancy of immunoreactivity to antigens present in liquid phase and antigens fixed in solid phase (tissue sections). Cuervo has developed a method for the immunocytochemical detection of transmitter substances or markers based on the use of internally labeled Mabs. A labeled amino acid, usually lysine, is added to the hybridoma

and becomes incorporated into immunoglobulin (IgG). In this way, Cuervo obtains a probe of high specific activity that is stable for up to 2 years. This method allows the demonstration of antigenic sites by light and electron microscopy radioautography (radioimmunocytochemistry). The second method takes use of bi-specific Mabs, a product of hybrid-hybridomas (e.g., substance P + peroxidase). This method permits the immunoenzymatic demonstration of antigenic sites using a single immunoglobulin (bi-specific Mabs).

A New Cholinergic-Specific Antigen. The characterization and distribution of a new cholinergic-specific antigen which they have termed Chol-1 was reported by E. Borroni, P. Ferretti, and J. Obrocki (Department of Neurochemistry, Max Planck Institute for Biophysical Chemistry, Göttingen). In a search for surface antigens specific for cholinergic neurons, Borroni et al. isolated the plasma membranes of the purely cholinergic electromotor nerve terminals of the electric organ (EO) of *Torpedo marmorata*. These plasma membrane fractions were used to raise an antiserum ( $\alpha$ -7SM) to them. The antiserum recognizes various gangliosides in *Torpedo* and cross-reacts with mammalian cholinergic terminals. This has mainly been demonstrated by its capacity to induce the selective, complement-mediated lysis of the cholinergic subpopulation of guinea pig cortical synaptosomes. *Torpedo* EO gangliosides were found to inhibit this lysis. Thus, the Chol-1 appeared to be gangliosidic in nature. This was tested by (1) a test of the inhibitory activity of guinea pig brain gangliosides after extensive purification or exposure to various biochemical treatments; (2) affinity purification of the  $\alpha$ -7SM on immobilized EO gangliosides and test of its activity in the lysis of cholinergic synaptosomes. Immunostaining on thin-layer chromatographic plates showed two bands corresponding to minor gangliosides migrating close to GT1b and GQ gangliosides. In addition, the affinity-purified serum was used to study the immunohistochemical distribution of Chol-1

in rat CNS. The results obtained indicate the existence of two polysialo-gangliosides bearing antigenic determinants specific for the cholinergic system.

#### Acetylcholine Receptor Blocking.

Studies using Mabs and antibodies from the serum of patients with myasthenia gravis to probe the binding sites for  $\alpha$ -bungarotoxin (BuTx) of the acetylcholine receptor (AChR) from *Torpedo* and from mouse muscle of 12 Mabs produced against the Bu-Tx-binding sites of the *Torpedo* AChR were reported by Z.W. Hall, A.J. Dowding, and Y. Gu (Division of Neurobiology, Department of Physiology, University of California, San Francisco). Two Mabs blocked Bu-tx binding completely, whereas the other Mabs caused 50 percent inhibition. The 10 that caused partial inhibition fell into two mutually exclusive groups. Inhibition caused by members of each group was not additive while members of different groups produced additive inhibition. Pharmacological experiments showed that the two groups of antibodies block pharmacologically distinct sites, with different affinities for d-tubocurarine (dTC) and for pancuronium.

Hall et al. also studied antibodies in a myasthenic serum that produce 50 percent inhibition of Bu-Tx binding and specifically block the high affinity site for dTC. These antibodies also inhibit the functional response of the receptor to acetylcholine. The relation between blockade of toxin-binding and inhibition of the functional response suggests that binding of antibody to a single toxin-binding site is sufficient to inactivate the receptor.

Isolation of Endogenous Benzodiazepine. The use of Mabs to isolate an endogenous benzodiazepine from mammalian brain was reported by A.L. De Blas (Department of Neurobiology and Behavior, State University of New York, Stony Brook). De Blas found that benzodiazepine-like molecules are present in selected neuronal populations of rat brain using the antibenzodiazepine Mabs 21-7F9. This finding was supported by immunocytochemistry and immunoblotting experiments.

The endogenous benzodiazepine-like material was isolated from bovine brain and purified to homogeneity by immunoaffinity chromatography on immobilized Mabs 21-7F9 followed by gel filtration on Sephadex G-25 and by two different high-pressure liquid chromatography (HPLC) steps. The purified substance has been identified as the benzodiazepene, N-des-methyl diazepam, using mass spectroscopy, HPLC, spectrophotometry, and BZDR-binding technique (inhibition of  $H^3$ -flunitrazepam binding to the neuronal-type benzodiazepine receptor).

Benzodiazepine-like immunoreactivity with the Mab 21-7F9 was also found in all the human brains tested including six brains that were stored in paraffin since 1940, 15 years before the first chemical synthesis of benzodiazepines. It appears that natural benzodiazepine-like molecules and possibly benzodiazepines themselves are present in human and other mammalian brains. However, De Blas does not know yet whether natural benzodiazepines or benzodiazepine-like molecules are biosynthesized by mammals or whether they are taken from the diet.

## 5 CONCLUSION

The greatly increased use of molecular biological techniques, including recombinant DNA methods to investigate problems in neurobiology which could not be approached with conventional biochemical methods was very evident in the presentations at the Sixth Meeting of the European Society for Neurochemistry. Furthermore, immunological approaches received a great deal of attention. The emphasis on the utilization of these powerful tools by European scientists is relatively recent since at the previous meeting of this organization held in Budapest, Hungary, in 1984 (ONRL report C-7-84), there was a paucity of presentations in this area. As in the U.S., many European scientists who had been trained as molecular biologists have entered the field of neurobiology to tackle research in this area at a truly molecular level.

## APPENDIX 1: SCIENTIFIC PROGRAM

### Plenary Lectures

- "Cloned Genes for Receptors," E.A. Barnard (UK).
- "Dynamics of Membrane Lipids: A Non-Equilibrium Model of Receptor-Ligand Interaction," L.D. Bergelson (USSR).
- "Receptor-Stimulated Inositol Phospholipid Hydrolysis and Neural Function," M.J. Berridge (UK).

### ESN Honorary Lectures

- "Covalently Attached Phosphatidyl-Inositol as a Hydrophobic Anchor for Acetylcholinesterase and Other Membrane Proteins," A.H. Futerman (Israel).
- "Neuronal and Extraneuronal Mechanisms of GABA in the Mammalian Periphery," S.L. Erdő (Hungary).

### Symposia

- "Molecular Aspects in Neurotransmitter Storage and Secretion," Chairmen: H. Winkler (Austria) and E. Neher (West Germany).
- "Molecular Mechanisms of Ischemic Brain Damage," Chairmen: B.K. Siesjö (Sweden) and J. Foebergrova (Czechoslovakia).
- "The Impact of Molecular Genetics on Neurochemistry," Chairmen: E.A. Barnard (UK) and U.Z. Littauer (Israel).
- "Receptors in Human Brain," Chairman: P. Laduron (Belgium).
- "Molecular Signalling for Growth and Differentiation in the CNS," Chairmen: W. Seifert (West Germany) and A. Schousboe (Sweden).

### Workshops

- "Receptor-Controlled Adenylate Cyclase," Chairmen: S. Hynie (Czechoslovakia), E. Costa (US), and M. Ui (Japan).
- "Progress in the Biology of Glial Cells," Chairmen: N. Baumann (France) and A. Prochiantz (France).
- "Functional Biochemistry of Brain Gangliosides," Chairmen: G. Tettamenti (Italy) and N.P. Avrova (USSR).
- "Neurotoxins and Ion Channels," Chairmen: J.O. Dolly (UK) and B.I. Khodorov (USSR).
- "Cerebrospinal Fluid Basic and Clinical Aspects," Chairmen: H. Link (Sweden) and J. Tichý (Czechoslovakia).
- "Neurotransmitters and Peptides in Stress," Chairmen: R. Kvetnansky (Czechoslovakia) and F.J.H. Tilders (The Netherlands).
- "Muscarinic Receptors," Chairmen: T. Bartfai (Sweden), M. Sokolovsky (Israel), and J. Järv (Estonian SSR).
- "Cell Recognition and Neuronal Choices in Neurogenesis," Chairmen: M. Weber (France) and E. Bock (Denmark).
- "Neuropathology of Axonal Transport," Chairmen: J. Sjöstrand (Sweden) and D. Tomlinson (UK).
- "Neurochemistry of Dementia," Chairmen: Y. Agid (France) and M. Shelanski (US).
- "Immunological Approaches to Synapsis and Development of the Nervous System," Chairman: A.C. Cuello (Canada).
- "New Approaches to Metabolic Regulation," Chairman: H.S. Bachelard (UK).
- "Phosphoinositides and Neurotransmission," Chairmen: S.R. Nahoraki (UK) and M.J. Berridge (UK).
- "Neuropeptides and Their Peptidases," Chairmen: A.J. Turner (UK) and I. Hajek (Czechoslovakia).
- "Na, K-ATPase and Its Physiological Regulators," Chairmen: P. Vyskocil (Czechoslovakia) and A. Swann (US).



#### Roundtables

- "Neurotransmitters in the Myenteric Plexus," Chairman: M.F. Shuka (USSR).  
"Substance P and Other Tachykinins," Chairmen: N.N. Osborne (UK) and M.R. Hanley (UK).  
"DNA Synthesis, Repair, and Degradation in the Brain," Chairmen: V. Mares (Czechoslovakia) and A. Giuditta (Italy).  
"New Trends in Extrapyrarnidal Disorders," Chairman: L.G. Battistin (Italy).  
"Regeneration Program of the Neuron," Chairman: G.W. Kreutzberg (West Germany).  
"Cholinergic Synaptic Vesicles," Chairmen: H. Zimmerman (West Germany) and S.M. Parsons (US).  
"Advances in the Isolation and Structural Analysis of the Opioid Receptors," Chairman: A. Borsodi (Hungary).

#### Poster Sessions

Acetylcholine Synthesis, Storage, and Release  
Acetylcholine Receptors and Cholinesterases  
Catecholamines and Serotonin  
Excitatory Amino Acids  
GABA and Benzodiazepines  
Synaptic Transmission and Synaptosomes  
Neural Peptides, Hormones, Proteins, and Polyamines  
Opioids and Substance P  
Myenteric Plexus  
Second Messengers and Protein Phosphorylation  
Membranes, Channels, Ions, and ATPases  
Axonal Transport and Peripheral Nerve  
Neuropharmacology and Neuropathology  
Extrapyrarnidal Disorders and Alzheimers' Disease  
Oxygen and Hypoxia  
Neuronal Regeneration and Transplantation  
Glia, Myelin, and Demyelination  
Lipids  
Nucleic Acids  
Neural Development  
Cerebrospinal Fluid and Blood-Brain Barrier  
Immunological Aspects  
Memory and Behavior  
General Metabolism